REMARKS

Claims 1-9, 12, 26-35, 38-50, 53-61 and 63-72 are pending in this application. Claims 26-35, 38-50, 53-61, 63-68 and 70-72 have been withdrawn from consideration as a result of the examiner's decision regarding the restriction requirement. Claims 1-9, 12 and 69 are under examination and have been rejected. By amendment above, claims 1, 2 and 8 have been amended, new claims 73 and 74 have been added and claims 5, 6, 16, 29, 46 and 60 have been canceled.

In paragraph 3 of the Action, the examiner has asserted that the Applicants have not complied with one of the conditions for receiving benefit of an earlier filing date under 35 U.S.C. §119(e). Specifically, the examiner noted that the application does not contain a specific reference to the prior application(s) in the first sentence of the specification. Applicants note that this oversight has been corrected in the amendment to the specification set forth above.

The drawings of the application also have been objected to on the basis that Figure 9 contains handwritten text. A replacement sheet with a corrected figure 9 is submitted herewith.

Claim 8 has been rejected under 35 U.S.C. §112, second paragraph, on the basis that the acronyms DOPE and MPB are not clear. Claim 8 has been amended to identify the terms. Both acronyms are commonly used in the art and are understood by persons of ordinary skill in the art.

Claims 1, 3-9 and 12 have been rejected under 35 U.S.C. §102(b) as anticipated by MacLean et al. as evidenced by Martin et al., Laukkanen et al., Gershon et al. and Lesoon-Wood et al. The examiner asserted that MacLean et al. teach cationic immunoliposomes comprising DNA wherein the immunoliposomes are targeted by the conjugated antibody/fragment thereof to a tumor cell. MacLean et al. were said to teach that succinimyl-maleimide cross-linkers could be used to attach antibodies/fragments thereof to liposomes and that Martin et al. teach the use of these cross-linkers to attach Fab' to liposomes. Laukkanen et al. were said to teach attachment of a ScFv mAb comprising a lipid tag to liposomes. Gershon et al. were said to teach different ratios of DNA to liposomes and that the resulting immunoliposomes comprising nucleic acid were highly efficient delivery vehicles for the DNA. Lesoon-Wood et al. were said to teach a lipid/DNA ratio of 11.4 nmol/1 ug. The examiner asserted that although none of the MacLean et al., Martin et al. nor Laukkanen et al. teach the ratio of protein: lipid recited in instant claim 1, Martin et al. do teach a ratio in concentration protein:umol lipid and resulting yields, and that Laukkanen et al teach the yield resulting from their process of preparing immunoliposomes is close to that taught by Martin et al. She further asserted that although none of MacLean et al., nor Martin et al. nor Laukkanen et al. teach the nucleic acid:lipid ratio recited in claim 1, Gershon et al. teach different ratios of DNA to liposome in ug of liposome to concentration of DNA, and Lesoon-Wood et al. teach a DNA/lipid ratio recited in claim 1. She concluded that the claimed complex appears to

be the same or similar to the process of the prior art absent a showing of unobvious differences. This rejection is traversed.

The primary reference cited by the examiner, the paper by MacLean et al., is a review paper. Beginning on page 328, the authors discuss methods of coupling antibodies to liposomes. They list seven different methods. In each of methods 1-6, the antibody is attached at the lipid headgroup region of the liposome; in the last method the antibody first is coupled to a polymer that will form complexes only with cationic liposomes and DNA. Applicants have amended claim 1 of their application to focus on a preferred method of making their immunoliposomes, in which the complex comprises an scFv antibody fragment conjugated directly to a reactive group on the liposome, the conjugation occurring through the covalent bonding of a sulfur molecule at the carboxy end of the antibody fragment, and in which the antibody fragment, liposome and DNA of interest are provided in specific amounts relative to one another. This method is not taught or suggested by MacLean et al.

The third method listed by MacLean et al., on which the examiner focused, is the use of succinimyl-maleimide crosslinkers to attach antibodies containing sulfhydryl groups to liposomes. The authors described this method as one in which the succinimyl end of the cross-linker reacts with $-NH_2$ groups on PE-liposomes, while the maleimide group reacts quickly and efficiently with sulfhydryl groups through the formation of a thioether bond. This is <u>not</u> comparable to the reaction method set forth in the present claims. The method referenced by MacLean et al. is a chemical reaction

in which an -SH group is introduced into a protein, in contrast to the presently claimed method in which an -SH group is introduced into the scFv via molecular engineering. Harsh chemical treatment as described in the paper referenced by MacLean et al. would damage or destroy the biological activity of the small scFv molecule.

Specifically, the method referenced by MacLEan et al. is described in detail in a paper by Duncan et al., a copy of which is provided with this response. In this method, the -SH is introduced by treatment with SATA (N-succinimidyl S-acetylthioacetate). As is explained in the accompanying Rule 132 Declaration submitted by Esther Chang, one of the co-inventors of the present invention, SATA can alkylate any exposed amino acid group in the protein and does so randomly. This results in what is known as a "Poisson distribution" of alkylated molecules, which means that only about 36% of the molecules are monosubstituted, 33% have multiple substitutions, and 31% are unsubstituted, which means that single substitutions can be obtained for only a small fraction of the IgG molecules. Furthermore, there is no way of directing the substitution to a specific amino acid. As Dr. Chang explains, this has two implications for scFv. First, the multiple substitutions form polymers which block the epitope on the scFv that binds to the receptor. In addition, even in the absence of such polymers, there is no way to direct which substitutions would bind to a reactive group, such as maleimide-DOPE, in the liposome. Thus, even if the antibody fragment was only monosubstituted, there is no guarantee that the correct amino acid would carry the substitution such that in the resulting complex the epitope that binds to the receptor would be exposed and

available for binding. The authors of the paper even recognize that their method does not work well for antibodies; on page 72 they suggest that "it is apparent that fewer potential polymerization sites will result if the single active group mentioned above is inserted into the peroxidase rather than the IgG" (emphasis added).

Both of these problems result in a loss of biological activity. This does not happen with the method used in the present invention to produce scFv-liposome complexes. The DNA encoding the scFv is engineered to produce a site that will provide a single -SH, the protein will refold properly to its natural state and the -SH will not interfere with the fragments' binding to its receptor.

It should be noted that none of the other methods of coupling antibodies to liposomes noted by MacLean et al. is suitable for directly coupling an scFv to a liposome, either. All of the methods require a whole antibody, a linker, and/or involve a chemical reaction that is unsuitable for use with scFv. The first method listed, biotin/avidin, is noted by MacLean et al. to be very inefficient. In addition, it requires a chemical reaction to attach a biotin to the NH_2 of the antibody, and one cannot direct where the biotin attaches. No guidance is provided for how one would use this reaction with any antibody fragment, much less an scFv. The second method also is acknowledged to be inefficient, and it also requires a chemical reaction involving multiple NH_2 groups that cannot be directed. This method, like the preceding one, is designed for use with whole antibodies, not antibody fragments. The fourth cited method, in which a pyridyl dithiopropionate group is reacted directly with an antibody, is

acknowledged by MacLean et al. to be little used as the resulting molecule is unstable. It also requires that the antibody have a free sulfhydryl group, which an scFv does not naturally have, as explained above (and there is no suggestion for how to provide such a group to scFv in the referenced method). The fifth listed method is useful only for whole antibodies, not fragments. The method was designed specifically for use with stealth liposomes, i.e., liposomes that have a PEG attached such that the antibody is attached to the end of the PEG. The sixth method cited requires that a hydrazide group reacts with an oxidized carbohydrate in the constant region of an antibody. The Fc region is not present in scFv. Finally, the last listed method requires the attachment of a poly-L-lysine with a terminal NH_2 group to an antibody. This method requires the use of a linker, not used in the present method of preparing immunoliposomes, and no guidance is provided for carrying out the method with antibody fragments.

The examiner asserted that MacLean et al. teach pharmaceutical compositions comprising nucleic acid - cationic liposomes and their use in treating cancer. This is not correct. The discussion regarding immunoliposomes and cancer begins on page 329 of the paper, and the opening sentence provides that cationic immunoliposomes "are still in the very early stages of development and to date no results have been reported" (emphasis added). None of the references cited by MacLean et al. teach the successful *in vivo* administration of immunoliposome-nucleic acid complexes to treat cancer, much less the successful *in vivo* administration of such a complex in which the

immunoliposome comprises an scFv antibody fragment directly attached to the liposome through a sulfhydryl group at the antibody fragment's carboxy terminus.

None of the secondary references cited by the examiner compensate for the deficiencies of the primary reference. The Martin et al. reference focuses on the coupling of Fab' fragments to liposomes. The claims of the present application focus on scFv antibody fragments, rather than Fab' fragments. One of skill in the art could not predict from this reference that immunoliposomes could be made comprising an scFV fragment rather than an Fab' fragment. An Fab is a naturally occurring fragment of a full antibody, separated from the Fc portion of the antibody by enzymatic digestion. An scFv, on the other hand, is a recombinant, engineered molecule in which the heavy and light chains of the antibody are separated and then linked with a small peptide. This is done at the DNA sequence level and the resulting expression vector is placed into a bacterial host. The protein expressed in isolated and purified, which includes the critical step of refolding the protein. If the protein does not refold properly, it loses its ability to bind to the receptor. At the time the present invention was first made, scientists had no assurance that the refolding would occur properly and bind as desired to the reactive group of the liposome such that biological activity would be retained.

Furthermore, Martin et al. teach a ratio of protein:lipid that is very different from that set forth in the pending claims of the present application. Martin et al. teach a ratio of 0.5 mg/1.4 umol protein:lipid to 4.0 mg/1.4 umol protein:lipid. Converting the ug/ug ratio set forth in claim 1 to ug/umol, the ratio claimed is 0.025 mg/1.4 umol to 0.2

mg/1.4 umol, which is completely outside the ratio taught by Martin et al. The present inventors use significantly less protein that Martin et al. found necessary. This illustrates a significant difference between using Fab' fragments and scFv fragments.

The Laukkanen et al. reference describes a different approach for attaching antibodies to liposomes other than the seven methods taught by MacLean et al. (MacLean et al. noted this further method at the end of their discussion of the seven "basic" methods for coupling antibodies to liposomes). The method described by Laukkanen et al., however, also is not relevant to the presently claimed invention. Laukkanen et al. teach attaching an sc Fv to a liposome through a lipid tag, which is quite different from the method of the pending claims. There are significant differences between a lipid-tagged (LPP) scFv and an scFv with a 3' terminal sulfhydryl group. LPP construction contains an amino acid linker sequence between the *E. coli* lipoprotein signal peptide (scLPP) and the scFv cloning site and an *E. coli* lipoprotein N-terminal nine amino acid sequence. Insertion of these sequences will lead to fatty acid acylation of the expressed signal in the *E. coli* host and its insertion into the bacterial membrane. Purification of the lipid-modified scFv sequence from the bacterial membrane results in an active molecule that can be inserted into the liposome. The teaching of the formation of a lipid-modified scFv sequence would not suggest that alternatively such an antibody fragment could be modified by engineering a sulfhydryl group to the carboxy terminus of the fragment which then could bond covalently to a reactive group on the liposome surface. It was unexpected that such engineering of an

antibody fragment would result in a protein that could be purified from bacterial inclusion bodies in sufficient quantities to be useful. It also was not predictable that the protein would refold properly to have biological activity, particularly after conjugation to the reactive group (maleimide) in the liposome. It further was unexpected that such a modified antibody fragment could bind directly to the liposome while maintaining binding activity. It was unknown, prior to the Applicants' invention, that one could introduce a sulfhydryl group, such as through the addition of a cysteine, to the 3' end of an scFv without negatively affecting the ability of the fragment to refold properly and maintain its biological activity. As there is no LPP in this molecule, it does not insert into the bacterial membrane, and its isolation and purification are very different from the that of the lipid-tagged antibody fragment taught by Laukkanen et al.

The third and fourth cited secondary references, the papers by Gershon et al. and by Lesoon-Wood et al., respectively, discuss liposome complexes that are composed solely of liposomes and DNA–the complexes do not comprise an antibody fragment, which is an essential component of the complexes of the present invention. Neither paper teaches or suggests antibodies or antibody fragments (including scFv) being used with cationic liposomes. The complexes they teach thus are quite different from those of the present invention. As the complexes contain no antibody fragment, there is no teaching or suggestion of a desired protein to lipid ratio, as is included in the pending claims. Lesoon-Woods et al. discuss a ratio of nucleic acid to liposome, but this is of little relevance in and of itself–the ratio of nucleic acid to liposome when

unliganded may well not be the optimal ratio when a protein is added. The ratio of protein to liposome also is important, and the optimal ratio of nucleic acid:liposome can be affected by the addition of the protein.

Thus, to summarize the inadequacies of the cited references, McLean et al. do not teach or suggest conjugating an scFv fragment to a liposome, much less achieving such conjugation through a sulfhydryl group at the 3' end of the fragment. They also do not suggest the ratio of protein:lipid and nucleic acid: lipid set forth in the claims. Martin et al. focus on immunoliposome complexes in which the antibody portion is provided by an Fab fragment, which is quite different from an scFv fragment. Neither the Gershon et al. or Lesoon-Wood et al papers discuss complexes which contain any form of antibody at all. Both of these papers focus simply on complexes of a liposome and nucleic acid. And finally, the Laukkanen et al. reference teaches scFv fragments which are prepared with a lipid tag for insertion into a liposome. Lipid-tagged antibody fragments are quite different from scFv fragments which have been genetically modified to provide a 3' sulfhydryl group which can bind directly to a reactive group in the liposome, and it was not obvious at the time of the present invention that the latter type of scFv could both bind directly to a liposome and retain biological activity upon doing so. Accordingly, the primary reference, as evidenced by the secondary references does not anticipate the presently claimed invention.

Claims 1, 3-8 and 12 have been rejected under 35 U.S.C. §102(e) as anticipated by U.S. Patent 6,071,533 (hereinafter referred to as the '533 patent). The examiner

characterized the '533 patent as disclosing nucleic acid-cationic immunoliposome complexes and pharmaceutical compositions thereof, wherein the nucleic acid could comprise DNA encoding p53. The examiner further asserted that the patent discloses that the targeting moiety (the antibody or antibody fragment) could be directly conjugated to the liposome through a thioether linkage (the antibody can be covalently bound to DOPE in the liposome linked to a sulfhydryl reacting group). The patent further was said to disclose that 1 ug DNA could be mixed with 5-15 nmol of lipid. She acknowledged that the reference does not teach the ratio of protein:lipid recited in claim 1, but she asserted that the complex appears to be the same as or similar to the complex of the patent. This rejection is traversed.

The '533 patent teaches an immunoliposome complex that is both very different from that claimed and prepared in a different way. The claims of the present application, as amended, focus on an immunoliposome complex comprising an scFv fragment which is directly linked to the liposome. In contrast, the '533 patent teaches complexes in which the antibody fragment is an Fab' fragment which is linked to the liposome through a hydrophilic polymer linker. There is no suggestion in the '533 patent that an scFv fragment could be used in place of the Fab' fragment, much less that such a fragment could be conjugated directly to the liposome. As has been explained above, in the discussion regarding the Martin reference, there are significant differences between the structure and behavior of Fab' fragment and scFv fragments. As of the priority date of this application, the direct conjugation of scFv to a liposome

was unknown, and one of ordinary skill in the art could not simply have substituted the scFv for an Fab' fragment. Furthermore, as scFv do not naturally possess a sulfhydryl group which can be conjugated to a reactive group on the liposome, that group had to be introduced through genetic engineering, and it was not obvious that the molecule would retain its biological activity. Fab' fragments, by contrast, can be provided with a free sulfhydryl group simply by subjecting the molecule to protease digestion.

The differences between the complex of the present invention and that of the '533 patent further is illustrated through the differences in activity of the complexes. The '533 patent provides in the paragraph bridging columns 23 and 24, and at the end of the last full paragraph in column 24, that the Fab-PEG-liposome/DNA complex is not active *in vivo* when the liposome comprises DDAB/DOPE ("DDAB/DOPE did not give any measurable activity," col. 24, lines 60-61). In contrast to this, the complexes of the present invention are active *in vivo* with these lipids. See Example 12 and Figure 9 of the present application (the liposome identified in the example as "LipB" is DDAB/DOPE, as identified in example 3 of the application). This difference in activity shows that the differences between the complexes claimed in the present application and those of the '533 patent are different and that the differences are significant. These differences are due to the way in which the complexes are formed: the present invention uses scFv with a sulfhydryl group at its 3' end, rather than Fab; the present invention does not include a polymer linker, as is required in the '533 patent; the order of addition of the components of the complex is different (in the presently claimed

invention, the scFv is mixed with the cationic liposome, then the resultant cationic immunoliposome is mixed with the nucleic acid of choice to form the final complex; in the '533 patent, the liposome is first mixed with the DNA of choice, the Fab fragment is mixed with the hydrophilic polymer linker, and then the two mixtures are mixed together). It was unexpected that these differences would result in complexes with different activities.

The '533 patent provide no *in vivo* data to show that the complex with Fab can specifically target a tumor and be used to treat patients. In Example 12 of the present application, however, such data are provided, and as noted above, the liposome used is DDAB/DOPE, the liposome which when complexed with the Fab fragment in the '533 patent was found to show no measurable activity upon administration. Furthermore, the only *in vivo* activity provided in the '533 patent is for complexes which did not contain the antibody fragment and were administered to non-tumor bearing mice. In that experiment, the majority of the complex went to the lung, which is not a good indication that the complex could be used to treat a variety of cancers.

Finally, the '533 patent does not provide any ratio of protein :lipid. The ratio provided in the claims of the present application is a key factor in providing a complex having a small size which can target tumors efficiently.

Claims 1, 3-9 and 12 have been rejected under 35 U.S.C. §102(e) as anticipated by U.S. Patent 6,210,707 (hereinafter referred to as the '707 patent). The examiner asserted that the patent discloses nucleic acid-immunoliposome complexes and

pharmaceutical compositions thereof, the complexes comprising cationic liposomes that have been coupled to antibodies or antibody fragments that target the liposomes to a specific cell type, and further comprising a nucleic acid molecule to be delivered to the specific cell type. The examiner further asserted that the targeting moiety, such as scFv, could be directly conjugated to the liposome by means well-known in the art, including covalently. The examiner acknowledged that the '707 patent does not disclose the w:w ratio of protein:lipid recited in claim 1, but asserted that it does disclose the ratio in ug protein:umol lipid and that the claimed complex appears to be the same or similar to that of the patent absent a showing of unobvious differences. This rejection is traversed.

The '707 patent is a CIP of the '533 patent discussed above. In this later patent, the inventors have included a discussion of scFv, as well as Fab, as the antibody fragment which can be used to make the immunoliposome complexes described, but the patent, like its parent, still provides no teaching or suggestion of how one could directly conjugate an scFv fragment to a liposome, as is taught and claimed in the present application. Instead, the '707 patent teaches complexes which require a polymeric linker to link the antibody fragment to the liposome.

In addition, contrary to the examiner's assertions, the ratio of protein:liposome is very different from that required by the pending claims. As noted above, the '707 patent teaches a ratio of scFv/liposome of 15.6 ug:1umol. In contrast, the pending claims require a w:w ratio of 1:5 to 1:40, which corresponds to 17.9 ug:1umol to 142.9

ug:1umol. Applicants have found that below the claimed ratios, the biological activity of the resultant complex is unsatisfactorily low; the targeting of the molecule to the target cell is inefficient.

Claims 1-8, 12 and 69 have been rejected under 35 U.S.C. §103(a) as unpatentable over Xu et al. in view of U.S. Patent 6,200,956 (hereinafter the '956 patent) and MacLean et al. Claim 9 was rejected under this same section of the statute over these same three references, taken further in view of U.S. Patent 4,946,778. As claim 9 has been canceled and the limitations of that claim have been included in claim 1, these two rejections will be addressed together.

The examiner asserted that the primary reference discloses transferrin-cationic liposomes mixed with DNA encoding wild type p53. She further asserted that the '956 patent generally discloses immunoliposomes covalently coupled to a ligand of a receptor present at the surface of a target cell type and further comprising DNA that is to be delivered to the said target cell type, and pharmaceutical compositions thereof and specifically discloses such immunoliposomes comprising antibodies or fragments of antibodies linked to the transferrin receptor. The examiner asserted that it would have been obvious to have used an antibody or fragment thereof to the transferrin receptor as disclosed in the '956 patent in place of transferrin in the nucleic acid-cationic liposome taught by Xu et al. She acknowledged that these references do not teach using a single chain antibody fragment, but asserted that the '778 patent discloses that such fragments are smaller, more stable and inexpensively produced.

She asserted that it would have been obvious to have used a single chain antibody fragment in place of transferrin in the nucleic acid-cationic liposome taught by Xu et al. This rejection is traversed.

As the examiner has recognized, the primary and secondary references do not teach or suggest immunoliposome complexes in which a single chain antibody fragment is covalently and directly linked to a liposome. There are other deficiencies in the primary and secondary references as well. The primary reference, the paper by Xu et al., discloses transferrin-liposome-DNA complexes. The complexes do not include an antibody or antibody fragment of any type and certainly there is no suggestion in the reference that an immunoliposome could be prepared in which an scFv fragment is conjugated directly to a liposome. Furthermore, the transferrin which is part of the complexes disclosed in the reference is a quite different molecule from the transferrin receptor single chain antibody fragment (TfRscFv) used in some of the examples of the present application and has quite a different function. Transferrin is a relatively large molecule (~80kDa) which transports iron; TfRscFv is an antibody fragment with a size of only ~28 kDa. In addition, the transferrin binds to the liposome through simple mixing; the TfRscFv (or other scFv selected) is chemically conjugated to a reactive group in the liposome.

In addition, the ratios of the components in the two complexes, one with Tf and one with TfRscFv as the antibody fragment, are quite different:

with Tf protein: DNA: liposome:Tf = 1 ug:8-10nmol:10-15 ug

with TfRscFv: DNA:liposome:TfRscFv = 1ug:6-20nmol:0.11-2.86 ug

(the amount of TfRscFv was determined by converting ug:ug scFv:lipid to ug:nmol, then calculating the range of protein at the 6-20 nmol liposome range using the TfRscFv:liposome range of 1:5 - 1:40 given in claim 1).

Thus, while the amount of liposome in the Xu et al. complex is similar to that set forth in claim 1 of the current application, the amounts of protein in the two complexes are very different. This difference illustrates that the ratio of liposome to protein can vary considerably depending upon the nature of the protein used in the complex and is important in ensuring that the complex will be effective *in vivo*. Two different types of proteins are not necessarily interchangeable at the same ratio.

Neither of the secondary references, taken individually or in combination, compensates for the deficiencies of the primary reference. The '956 patent focuses on a composition comprising a nucleic acid, a transfection agent and a compound involved in the condensation of the nucleic acid; the compound derived from a histone, a nucleoline, a protamine and/or a derivative thereof. The transfection agent can be a liposome. The patent provides that the composition further can comprise a targeting element to direct the transfer of the nucleic acid, the targeting element being linked to the compound or to the nucleic acid. The patent lists a variety of elements that can comprise the targeting element, including sugars, peptides, hormones, vitamins, cytokines, oligonucleotides or lipids, and states that examples of target element peptides are antibodies or antibody fragments. That is the sole reference to antibodies

in the specification. The patent makes no reference to scFv, and it certainly does not teach or suggest genetically modifying an scFv such that it has a 3' sulfhydryl group and then covalently binding such an scFv to a reactive group in a liposome.

The deficiencies of the MacLean et al. paper have been discussed above, and that discussion is equally applicable to the present rejection. MacLean et al. do not teach or suggest how to modify an scFv such that it can be covalently linked to a liposome.

The general disclosure of the uses of scFv fragments in the '778 patent does not overcome the deficiencies of the other references and is insufficient to render the invention set forth in claim 9 obvious. The replacement of a protein, such as transferrin, in a protein-liposome complex with a single chain antibody fragment, such as a TfRscFv, was not a matter of a simple replacement. There is nothing in the'778 patent which teaches or suggests how to directly covalently bind an scFv to anything, and certainly not to a lipid or liposome. As Applicants have explained above, such binding could be achieved only after they had genetically modified the 3' end of an scFv to provide a sulfhydryl group that could bind directly to a reactive group of a liposome. Such a modification is not taught or suggested in the art and would not have been obvious to do, as there was no certainty that the resultant fragment would refold properly and maintain its activity. Nowhere in any of the cited references is there a discussion or suggestion of how to link an scFv directly to a liposome without the aid of a polymeric linker.

In addition, although the patent discusses using such fragments to deliver drugs, there is no disclosure or suggestion that such fragments could be used to deliver DNA to cells.

In view of these deficiencies, the combined teachings of the cited references do not suggest the immunoliposome complexes claimed in the present application.

Claims 1-9, 12 and 69 have been rejected under 35 U.S.C. §103 (a) as obvious over MacLean et al., Martin et al., Laukkanen et al., Gershon et al. and Lesoon-Wood et al. in view of the '956 patent. MacLean et al. were cited as teaching cationic immunoliposomes comprising DNA which are targeted by the conjugated antibody or antibody fragment to a tumor cell. Martin et al. was cited for teaching the coupling of Fab' fragment to liposomes within certain ratios. Laukkanen et al was cited as teaching the attachment of an scFv mAb comprising a lipid tag to liposomes. Gershon et al. was cited as teaching different ratios of DNA to liposome and that the complexes formed between cationic liposomes and nucleic acid molecules are highly efficient vehicles for the delivery of nucleic acid. Lesoon-Wood et al were said to teach a DNA :lipid ratio within the range recited in claim 1 of the present application in a liposome comprising DNA encoding wt p53. The '956 patent was cited as teaching immunoliposomes comprising DNA to be delivered to a target cell type and as teaching that antibodies or antibody fragments to the transferrin receptor can be used to target the liposomes for cells such as tumor cells. The examiner asserted that it would have been obvious to use the ratios taught by Martin et al., Laukkanen et al. Gershon et al. and Lesoon-

Woods et al. in the nucleic acid-cationic immunoliposomes taught by MacLean et al. and to have used the antibody fragment that can bind to the transferrin receptor as disclosed by the '956 patent. This rejection is traversed.

The combination of the Maclean et al., Martin et al., Laukkanen et al., Gershon et al. and Lesoon-Wood et al. references, and their deficiencies have been discussed above. The addition of the teachings of the '956 patent do not compensate for the deficiencies of these references. As discussed above, none of the first five references teaches or suggests the immunoliposome complexes in which an scFv fragment has been directly covalently linked to a liposome. One could not simply substitute such a fragment for a whole antibody or an Fab' fragment in an immunoliposome containing the same, as the scFv fragment does not naturally contain a group at its 3' end which can bind to the liposome. It was Applicants' discovery that the sequence encoding such a fragment could be molecularly modified to introduce a group that upon expression of the fragment could provide a site for direct linking to a reactive group on the liposome without destroying the fragment's ability to properly refold or its biological activity.

The '956 patent does not overcome the deficiencies of these references. As previously explained, the '956 patent also does not teach or suggest immunoliposomes in which an scFv is directly bound to the liposome. The focus of the '956 patent is a composition comprising a nucleic acid, a transfection agent and a compound involved in the condensation of the nucleic acid; the compound derived from a histone, a nucleoline, a protamine and/or a derivative thereof. The transfection agent can be a

liposome. The patent lists a variety of targeting elements which can be included in the compositions to direct the transfer of the nucleic acid, including antibodies or antibody fragments, but there is no reference to scFv, much less a teaching or suggestion of genetically modifying an scFv such that it has a 3' sulfhydryl group which can bind directly to a reactive group in a liposome.

Furthermore, the references do not teach or suggest the specific ratios of protein, liposome and nucleic acid required by the present claims. Again, the shortcomings in this regard for the first five of the cited references has been discussed above, and the '956 patent does not compensate for those deficiencies. As the patent does not suggest immunoliposome complexes comprising scFv directly bound to a liposome, it clearly cannot suggest the ratios of protein, liposome and nucleic acid set forth in claim 1.

Claims 1-8, 12 and 69 have been rejected under 35 U.S.C. §103(a) as unpatentable over the '533 patent in view of the '956 patent. The examiner asserted that it would have been obvious to used the antibody to transferrin disclosed in the '956 patent in the invention of the '533 patent. This rejection is traversed.

The shortcomings of both of these references have been outlined above, and those discussions are equally applicable to the present rejection. Neither reference teaches or suggests a cationic immunoliposome complex which comprises an scFv fragment directly covalently bound to a liposome.

Claims 1-9, 12 and 69have been rejected under 35 U.S.C.§103(a) as unpatentable over the '707 patent in view of the '956 patent. Both of these references were cited for the reasons given in preceding rejections. This rejection is traversed.

The deficiencies of both of these references have been discussed in response to other rejections and those comments are equally applicable to the present rejection. The '707 patent does not teach or suggest how to directly bind an scFv to a reactive group of a cationic liposome. The scFv used in that patent is bound to a liposome through a polymeric linker. In addition, the ratio of protein:liposome is very different from that required by the pending claims. As noted above, the '707 patent teaches a ratio of scFv/liposome of 15.6 ug:1umol. In contrast, the pending claims require a w:w ratio of 1:5 to 1:40, which corresponds to 17.9 ug:1umol to 142.9 ug:1umol.

As has been explained above, the '956 patent does not teach or suggest the linking an scFv to a liposome--either directly or through a linker. It makes no reference to scFv at all.

Claim 9 has been rejected under 35 U.S.C. §103(a) as unpatentable over the '533 patent in view of the 956 patent and further in view of the '778 patent. The examiner acknowledged that the '533 patent and the '956 patent do not teach using a single chain antibody fragment, but she asserted that the '778 patent does disclose such fragments and that it therefore would have been obvious to have used such fragments in complexes with liposomes as taught by the other references. By amendment above, claim 9 has been canceled and its limitations incorporated into

claim 1. The rejection will be discussed with regard to claim 1 and the claims dependent upon it.

As the examiner recognized, the '533 and '956 patents do not teach or suggest the use of single chain antibody fragments in immunoliposome complexes. The '778 patent does not provide the guidance necessary to be able to directly conjugate such a fragment to a liposome. As has been discussed above, the replacement of a protein, such as transferrin, in a protein-liposome complex with a single chain antibody fragment, such as a TfRscFv, was not a matter of a simple replacement. There is nothing in the'778 patent which teaches or suggests how to directly covalently bind an scFv to anything, and certainly not to a lipid or liposome. As Applicants have explained above, such binding could be achieved only after they had modified the 3' end of an scFv to provide a sulfhydryl group that could bind directly to a reactive group of a liposome. Such a modification is not taught or suggested in the art and would not have been obvious to do, as there was no certainty that the resultant fragment would refold properly and maintain its activity. Nowhere in any of the cited references is there a discussion or suggestion of how to link an scFv directly to a liposome without the aid of a polymeric linker.

In view of the foregoing amendments and discussion, applicants respectfully submit that the pending claims of the application are in condition for allowance.

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